ACTIVE IMMUNIZATION AGAINST POLIOMYELITIS*

HERALD R. COX, ScD†

Mr. President, Fellows, and guests of the Academy, Dr. Hammon has just told you of some of the beneficial aspects, as well as some of the drawbacks, of gamma globulin (immune serum globulin) therapy, and I am sure that we most certainly agree with Dr. Hammon that immune serum globulin is not the final answer to the problem of long-term protection against poliomyelitis. The various shortcomings of immune serum globulin therapy have been discussed in considerable detail elsewhere,[1] so that it is not necessary to discuss that phase of the problem at this time. Without doubt, the only approach to the poliomyelitis problem is to bend every effort to develop a safe and practical immunizing agent: a vaccine that will give long-lasting protection against the disease. To that end, we have the possibility of developing either a killed preparation or a living attenuated virus vaccine.

Other investigators, such as Morgan,[2] have shown that monkeys inoculated repeatedly (12 to 15 times) with inactivated poliomyelitis virus—infected spinal cord tissue of monkeys treated with formalin—produce neutralizing antibody in fairly high titer and show measurable resistance to challenge with virulent virus. Recently, Howe[3] has reported some preliminary trials carried out in man with inactivated vaccines prepared from spinal cords of infected monkeys. Killed vaccines of this type, prepared from infected central nervous tissue, may be dismissed immediately from practical consideration, first, because poliomyelitis virus cannot be concentrated and readily freed of the nervous tissue components that are responsible for producing allergic encephalitis; second, it is doubtful

*Presented at the Stated Meeting of The New York Academy of Medicine in the Symposium on Poliomyelitis: Its Control and Prevention, April 2, 1953; manuscript received June 1953.

†At the time this paper was written, Dr. Cox was Director, Viral and Rickettsial Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

that enough monkeys are available to produce such a vaccine in the necessary quantities. I might add that the two primary factors that caused us[4–10] to develop the living avianized rabies vaccine were (1) we were not able to develop a satisfactory method of freeing brain tissue vaccines of the factor or factors responsible for producing allergic encephalitis and still retain a stable and potent product, and (2) we wished to provide longer-lasting protection than that usually afforded by killed vaccine. Common sense dictates that we should profit somewhat at least from the sad experiences we have had with rabies vaccines prepared from nervous tissues and not risk inducing the same paralytic accidents with a vaccine for poliomyelitis.

Recent advances in the field bear great promise that, in the foreseeable future, a method will be developed by which we can produce a statisfactory vaccine for protection against poliomyelitis. One of these developments is the work of Enders and his associates,[11–19] who first showed that it is possible to grow all three types of poliomyelitis virus in cultures of non-neural tissues of human origin. This procedure, of course, obviates the risk of causing allergic encephalitis since non-nervous tissues such as kidney, uterus, or muscle are used. This approach for solving various aspects of the poliomyelitis problem, such as virus detection, propagation, assay, antibody quantitation, and preparation of diagnostic antigens and of vaccines has been pursued by numerous workers in the field, including Salk and his associates at the University of Pittsburgh,[20–22] Melnick at Yale,[23–27] Syverton at the University of Minnesota,[28–32] Rhodes at the University of Toronto,[33–35] and Evans at the Univeristy of Washington.[36,37]

At this time, I wish to point out that any vaccine prepared from tissue cultures that contain mammalian cells taken at surgery or from sacrificed animals must of necessity be a killed vaccine. Because of the inherent danger of picking up extraneous contaminant viral, rickettsial, bacterial, or carcinogenic agents, it would be unwise and impractical for anyone charged with the responsibility of commercial vaccine production to assume the risk of producing a living, modified virus vaccine from explanted tissues derived from a mammalian source.

Recently, Salk[38] reported preliminary experiments in which he vaccinated approximately 161 human volunteers with formalin-killed virus vaccines prepared from tissue culture materials using either minced monkey kidney or monkey testicle. He found that, if given intradermally as an aqueous preparation, the vaccine induced an antibody response only against the Lansing Type 2 virus. A water-in-mineral-oil emulsion type of killed vaccine prepared from all three types of poliomyelitis virus (Brunhilde, Lansing, and Leon) was claimed to induce antibody response against all three types when the vaccine was administered

intramuscularly. The antibody levels of the vaccinated subjects were determined by tissue culture technique.

It should be noted, however, that all antibody titers reported were from sera taken a very short time after vaccination, that is, not longer than four to seven weeks postvaccination. Furthermore, significant antibody levels were found in most of the people *prior* to vaccination, namely, in 6 of 12 against Type 2, 7 of 16 against Type 3, and all 15 of 15 against Type 1 poliomyelitis virus. It must be realized, of course, that it is much easier to demonstrate the antigenic booster effect or anamnestic response to an antigen by vaccinating individuals who have had a previous experience (immunizing infection) with the antigen than it is to stimulate antibody production in persons who have never had any previous exposure to the agent. Thus critically reviewed, Salk's results are not too impressive.

Salk also indicated that mineral oil adjuvant was necessary to make the issue culture virus antigens become immunogenic, and that vegetable oils could not be used as a substitute for mineral oils.[39] It is difficult at the moment to evaluate fully Salk's results for many of his experiments were not completed when his report was published. However, I believe it is not out of order to utter a word of caution about certain potential complications that may arise from such a vaccine preparation. While animal experiments have failed to demonstrate any carcinogenic action of certain highly refined paraffinic mineral oils,[40] especially Russian or Pennsylvania oils, which are essentially free of aromatic compounds,[41] there is always the possibility that malignant tumors might occur in particularly susceptible individuals. Furthermore, it should be remembered that mineral oils are not saponifiable, and that they behave essentially as a foreign body in the tissues of the host. They are not hydrolyzed, but remain unchanged and physiologically inert for long periods of time, probably for life, regardless of how administered. The reactions to such oils have been described as foreign body granulomata, benign oil tumors, oleomas, or oil cysts, which sometimes result in slow-healing abscesses.[42,43]

It should be noted that the surest way apparently to determine carcinogenicity of an oil is to inject it into a large number of mice, rats, and rabbits intramuscularly, once a month for at least three months, and observe the animals for the rest of their natural lives. In extensive experiments by this procedure, Hueper[44] has demonstrated sarcoma formation with oils that were entirely noncarcinogenic when applied to the skin. It is evident that it would be most difficult, if not impossible, for a commerical biological laboratory to carry out such tests on each and every lot, or production batch, of oil used for vaccine preparation. Yet, no

one can deny the fact that such tests should be carried out if the product is to be considered safe.

In any case, the oils remain in the tissues for years, and in individuals who give an extensive foreign body response, surgical removal of the tumors may be required. It is possible that killed vaccines that contain mineral oil as an adjuvant will give stronger and longer-lasting immunity than killed vaccines in an aqueous base, yet it is most doubtful that either will give as long-lasting immunity as will a living, attenuated virus vaccine.

It probably will be necessary to revaccinate with the killed vaccines every two to three years in order to maintain the desired state of protection. When one considers that the product will be used primarily in young children and that the mineral oil component of each injection may persist in the tissues for life, it at once emphasizes the need to proceed with caution. Any procedure that requires repeated vaccinations over the years greatly increases the chances of sensitizing the individual to foreign proteins injected and cannot be considered as the ideal solution to the problem at hand.

In other words, the mineral-oil-adjuvant vaccine approach *may be* a safe procedure, but that can be proved only over a long period of time and should be determined only with a limited number of vaccinated persons. Certainly, it will take much more time to evaluate the safety of a mineral-oil-adjuvant-type vaccine than it will a living, attenuated virus vaccine. It would prove to be most embarrassing to have a situation develop comparable to that encountered with killed rabies brain tissue vaccines by which the vaccination procedure actually causes a greater number of deaths or unfortunate sequelae than occur among the unvaccinated who have been exposed similarly.[45,46]

Another approach toward solving the problem is to secure in tissue culture a pure line strain of normal mammalian cells, either human or animal in origin, proven to be free of any extraneous viral or bacterial contaminants and known to possess no carcinogenic activity. If it were possible to maintain such a pure line strain of normal cells in tissue culture and to propagate the cells in large enough quantities to meet our research and production needs, we would have a very useful medium. Unfortunately, at the moment we do not have the necessary information to accomplish this task, but the problem is worthy of a great deal of attention and energy.

By using a stable strain of human malignant epithelial cells (Héla strain), derived from an epidermoid carcinoma of the cervix, for the cultivation of all three types of poliomyelitis viruses, Syverton and his associates[32,47] have been able to quantitate antibodies by the neutralization technique to produce virus in

quantity in serial passage and to isolate and type viruses rapidly. This is a significant contribution, but it is indeed unfortunate that the Héla strain is of neoplastic origin. Of course, it would be the medial discovery of the century if it were found that a chemically inactivated poliomyelitis vaccine prepared from tissue culture cells of the Héla type was carcinogenic, but that potential danger must be considered. Thus, at the moment at least, it is more doubtful that anyone would recommend the use of neoplastic cells in tissue culture as the medium for producing poliomyelitis vaccine.

Before proceeding, I wish to emphasize that the tissue culture technique is an extremely important tool, especially for research purposes, for which it can be applied to so many problems, such as isolation, identification, and propagation of infectious agents; quantitation of antibodies; and assay of infectious agents by the neutralization technique; and for the development of virus mutants or variants that may be adapted to new and ordinarily nonsusceptible hosts. I might add that we are interested particularly in this last application of the technique because it has unlimited possibilities. Just recently, Li and Schaeffer reported[48] an important application of this procedure to establish the Brunhilde strain of poliomyelitis virus in albino mice.

I believe it is appropriate to note at this point that tissue culture methods have been recognized and previously used to good advantage to solve other difficult problems. The highly dramatized problem of poliomyelitis is not the first instance in which tissue culture procedures have been applied successfully in order to get a "foot in the door." As a matter of fact, both Theiler[49–51] and I[52–58] used tissue culture procedures as research tools or intermediate steps in the development of the 17D living virus, yellow fever vaccine, and the killed rickettsial vaccines against Rocky Mountain spotted fever, epidemic typhus fever, and Q fever that are being produced commercially today.

Theiler and Smith[49,50] used cultures consisting of minced chick embryo containing minimal amounts of nervous tissue to procure the 17D variant of the Asibi strain of yellow fever, which is used today as living attenuated virus vaccine. A similar mutant strain of yellow fever apparently was obtained subsequently by Penna and Moussatché.[59] I used cultures consisting of various tissues of the developing embryonic chick to determine that the yolk sac is quite superior to other tissues for growing all types of rickettsiae in large quantities.[52,54,55,58] This observation led to the yolk sac method of inoculation of fertile hen's eggs that is used today for the propagation of practically all viral and rickettsial agents.[60]

The tissue culture method was shown previously to be more sensitive than

animal inoculation for detection of minimal quantities of at least one other neurotropic virus. Thus, in 1936, I[61] reported that the inoculation of tissue culture preparations was a considerably more sensitive method for detection of minimal quantities of Eastern equine encephalomyelitis virus than was the usual procedure of inoculating guinea pigs or mice intracerebrally. So far as I know, the only vaccines being produced on a comparatively large scale by tissue culture methods are those against foot-and-mouth disease, developed by Frenkel,[62–66] and hog cholera, developed by Boynton.[67,68]* It should be noted that both these vaccines are used only in the field of veterinary medicine, and that one is a killed vaccine (foot-and-mouth disease), while the other is a living virus preparation that is used in conjunction with immune serum (hog cholera).

Still another approach toward solving the poliomyelitis problem is to develop a living modified virus vaccine. Without doubt, the most practical and greatest success in immunizing man and animals against viral infections has been achieved thus far with the use of living modified or attenuated viruses. In the case of human infections, I point out specifically smallpox, yellow fever, and rabies; in the case of veterinary medicine, I mention rinderpest, South African horse sickness, blue tongue of sheep, fowl pox, pigeon pox, infectious laryngotracheitis of chickens, and the new products that recently have been developed and produced in my laboratory, such as "wing web" vaccine and "intranasal" or "conjunctival" vaccine for Newcastle disease,[69–72] chick-embryo-propagated distemper vaccine,[73–78] chick-embryo-propagated rabies vaccine,[4–10] and the rabbit-adapted hog cholera vaccine.[79–82]

Returning to the problem at hand, namely, poliomyelitis, a review of the literature indicates that the two most promising paths for inducing immunity are the intramuscular and oral routes. Further, evidence obtained in the use of

*By the standards of a tissue culture specialist, his method is comparatively crude, but Frenkel has introduced some very ingenious time- and labor-saving devices that give a practical tissue culture medium for producing foot-and-mouth vaccine against at least three immunologically distinct types of the virus (European A, O, and C types), using explanted epithelial tissue of the bovine rumen or tongue. Frenkel's problem has been somewhat simplified in that maximal growth of the virus (titers of 10^{-6} or greater) is obtained within 18 to 24 hours. This, of course, greatly facilitates the suppression of bacterial contaminants by using antibiotics or sulfonamides. Frenkel's final product is a formalin-killed vaccine. Boynton used swine bone marrow or other swine tissue explants suspended in suitable physiological media. The hog cholera virus was presumably attenuated for swine by prolonged passage in tissue culture and is not produced commercially. The product recommended for field use consists of simultaneous injections of living virus propagated in tissue culture and immune serum, thus indicating that the cultured virus may be partially, but not completely, modified in its virulence for swine.

other living virus vaccines strongly indicates the importance of using the natural portal of entry whenever possible to achieve maximal beneficial effects of the immunizing agent.

We now recognize that large epidemics of paralytic poliomyelitis in certain countries are apparently a reflection of improvement in sanitary conditions. By improving our living habits, we have altered or upset nature's balance so that, instead of having a symbiotic relationship between the host and the infectious agent, we actually have created a nonimmune population in which large epidemics of paralytic poliomyelitis can be expected and do occur. In those parts of the world that have not improved their living and sanitary habits, poliomyelitis epidemics are apparently rare and relatively unimportant, and paralysis, when it does occur, is almost always limited to children of the younger age groups. As the virus is very widely distributed in these so-called underprivileged areas, most people come in contact with it while they are quite young and progressively acquire their immunity, in all probability, through infection by way of the oral and gastrointestinal routes, so that paralytic poliomyelitis in these countries is comparatively rare.[83] I am of the opinion that probably the most logical and practical way to immunize infants and children against poliomyelitis is to follow the pattern that seems to take place so universally under natural conditions, that is, to use an attenuated, living virus under biologically and quantitatively controlled conditions by a natural portal of entry—the oral route.

I believe that the studies carried out in our laboratory with the intranasal or conjunctival type of Newcastle disease vaccine have served as a very useful model for much of our poliomyelitis work. Thus, my colleagues Drs. Markham and Bottorff[71,72] have demonstrated that by using the proper quantitative conditions, a single drop of the relatively avirulent Blacksburg strain of Newcastle disease virus will readily immunize either fully susceptible or passively immune one-day-old baby chicks, provided the portals of entry of natural virus infection, namely, the intranasal or conjunctival routes, are used. Such vaccinated birds show no signs of illness following vaccination and often fail to develop any appreciable antihemagglutinins, particularly if the virus menstruum possesses no unusual surface activity; yet, they will readily withstand intranasal challenge of at least a million lethal doses of highly virulent field strains of virus 7 to 10 days and 5, 7, and 10 weeks later. Surface-active agents, which possess no antiviral activity, greatly enhance the serological response induced by intranasal vaccination. In contrast, a negative or irregular immune response is obtained when the same or much greater quantity of the Blacksburg strain of virus is administered to passively immune birds via unnatural portals of entry, such as the intraperitoneal,

subcutaneous, or intramuscular routes. The receptor cells of the respiratory tract, the natural portal of entry, are free to receive the virus introduced regardless of the state of passive immunity of the host. The virus becomes established and multiplies sufficiently to create a local barrier of actively immune cells at the portal of entry. A period of 7 to 10 days is required for birds to become immune using the Blacksburg strain of virus under these conditions. During this period, the birds should be kept in clean quarters, free from virulent strains of Newcastle disease virus, because no appreciable cell-block or interference effect is produced by the Blacksburg strain of virus when used in this manner. This strain is of such low virulence, or spreading potential, that contact infection is ordinarily not transmitted from vaccinated to fully susceptible, unvaccinated chicks housed in the same brooder. After the immune state has become established, the vaccinated birds are able to withstand tremendous doses of extremely virulent viral suspensions introduced either intranasally or in the conjunctivae.

To date, more than 460 million birds have been vaccinated with this product. The birds, in spite of their systemic immunity, obviously sustain at least a localized, clinically inapparent infection following challenge because they show very marked rises in antibody levels. This mechanism of inducing initial, basic immunity followed by "booster" challenges of living virus via a natural portal of entry seems to be paralleled in nature by infection with poliomyelitis virus. To achieve successful immunization against poliomyelitis, the goal is to duplicate the processes of natural infection under biologically and quantitatively controlled conditions.

As early as 1934, Kolmer and Rule[84] fed living poliomyelitis virus to rhesus monkeys in an attempt to immunize them. Unfortunately, an immune response was not engendered because the wrong experimental host was selected for these early studies. Recently, Koprowski, Jervis, and Norton[85] carried the work a step further by feeding human volunteers a living, rodent-adapted poliomyelitis virus. Briefly, the virus consisted of a suspension of brain and spinal cord tissues of cotton rats infected with the TN strain, Lansing type, poliomyelitis virus. A total of 20 children were fed dosages ranging from 1 ml to 10 ml each of the cotton rat tissue suspensions. All individuals were observed carefully, but none showed any signs of illness nor were any temperature rises noted. Most of the subjects became intestinal carriers for a short time of the virus strain that was fed. All subjects that were not initially immune developed neutralizing antibodies to the homologous Lansing-type virus, but not to the heterologous Brunhilde-type virus. Further work carried out by my laboratory in conjunction with the California State Department of Public Health, the California State Department of Mental

Hygiene, and the George Williams Hooper Foundation of the University of California has confirmed and somewhat amplified the above studies.[86]

While I subscribe fully to the thesis that the use of living, attenuated virus offers the most logical and hopeful approach toward solving the poliomyelitis problem, I am of the opinion that the virus should not be propagated in infected mammals or in infected mammalian explanted tissues until other approaches have been exhausted. Those reponsible for producing vaccines commercially recognize that such infected mammalian tissues are not ideal to use because they always present the danger of being contaminated with other viruses or microbic agents that are infectious for man. These include lymphocytic choriomeningitis, infectious hepatitis, Sabin's B virus, encephalomyocarditis viruses, and so on.

I believe that the poliomyelitis problem would be greatly simplified if the developing chick embryo could be used as the propagating medium since it has proved to be so nearly the ideal host for mass producing many other living attenuated viral vaccines, such as yellow fever, smallpox, rabies, blue tongue of sheep, rinderpest, Rift Valley fever, Newcastle disease, canine distemper, fowl pox, pigeon pox, and infectious laryngotracheitis of fowls, and killed viral and rickettsial vaccines, such as Rocky Mountain spotted fever; epidemic and murine typhus fever; Q fever; influenza; mumps; Western, Eastern, and Venezuelan equine encephalomyelitis; and Japanese B encephalitis.[60] The chick embryo possesses the admirable qualities of always being readily available, easily handled, relatively cheap, and, above all, free from microorganisms potentially dangerous for man. Furthermore, the chick embryo has a marked advantage in that it is not a natural host for poliomyelitis virus, and if a mutant strain can be established, there is very little likelihood it will regain its virulence for man.

Until quite recently, poliomyelitis virus was one of the comparatively few viruses that had resisted cultivation in developing chick embryos. However, during the past year workers in my laboratory have demonstrated that at least one strain of poliomyelitis virus can be cultivated in this host.[1,87,88] This work was started four to five years ago by Dr. Moyer and myself[89] in an attempt to develop a more-practical method for producing complement-fixation diagnostic antigens. By carrying each of two sublines of the virus (A and B series) through more than 150 serial transfers in suckling hamsters, using the intracerebral route of inoculation and infected brain tissue for passage, Moyer[89] brought about a modification of the MEF1 virus. By continuing the hamster passages over an approximate four-year period, the virus content in suckling hamster brain tissue eventually reached LD_{50} titers of $10^{-6.5}$ to $10^{-7.0}$ or greater, as determined by intracerebral titrations in white mice. The high titer secured in the suckling hamster brain

tissues encouraged us to renew out attempts to establish poliomyelitis virus in developing chick embryos.

Dr. Roca-Garcia[87] established the 119th hamster passage of the MEF1 strain of poliomyelitis virus in chick embryos via the yolk sac route of inoculation. Dr. Cabasso[88] shortly thereafter confirmed Dr. Roca's findings by establishing the 131st hamster passage virus in chick embryos by the allantoic cavity route of inoculation. Since establishing the original strains in the chick embryo, we have reinvestigated frozen, infected brain tissues stored at –70°C and have succeeded in establishing the 115th hamster passage of the MEF1 strain poliomyelitis virus in developing chick embryos.

The adapted strain of poliomyelitis may be readily maintained and propagated in the developing chick embryo. Present evidence indicates that the major changes effected in the characteristics of the virus are induced by adaptation to the rodent host, and that these characteristics remain essentially unchanged in the avian host for a considerable number of passages at least. One of the peculiar properties of the adapted MEF1 strain of poliomyelitis virus is that the chick embryo shows little, if any, signs of infection. The embryos do not die from acute infection, as is so common with many other neurotropic viruses, but the great majority die at the time just prior to hatching. Maximal growth of the virus takes place apparently in the embryo itself, and the optimal time to harvest the infected tissues is on the fourth to fifth day following inoculation.[90,91]

At present, we are intensively engaged in studying the properties of the various substrains of the chick-embryo-adapted virus in relation to their safety, as well as to their immunizing capacity for primates—particularly rhesus and cynomolgus monkeys and chimpanzees.

Table I illustrates some of the more pertinent data concerning some of the experimental vaccines prepared from infected chick embryo tissue and the reactions induced in rhesus monkeys inoculated intracerebrally. It must be realized, of course, that the data presented are still quite preliminary in nature. The vaccines used consisted of 20% chick embryo tissue homogenized in distilled water. They are being studied for their degree of pathogenicity for rhesus monkeys inoculated intracerebrally with 0.5-ml amounts of undiluted vaccine or serial dilutions thereof. A series of vaccines was prepared from the 17th, 20th, 30th, and 40th serial passages in chick embryos derived from the 131st passage in suckling hamsters (Moyer's A series[89]). The B series of vaccines was prepared from the 10th and 20th serial chick embryo passages derived from the 127th passage in suckling hamsters (Moyer's B series[89]). It is noted that the LD_{50} titers of these vaccines, determined by intracerebral titration in mice, vary from $10^{-3.66}$

TABLE I. MEF1 Poliomyelitis Chick Embryo (20%) Intracerebrally in Rhesus Monkeys

Vaccine	No. of Monkeys	LD_{50} Titer in Mice	Reaction	
			Negative	Positive
A17	30	$10^{-5.00}$	28	1+ (10*); 1++++ (15)
A20	10	$10^{-4.54}$	7	1+ (16); 1++ (9); 1++ (28)
A30	10	$10^{-3.83}$	9	1+ (18)
A40	10	$10^{-3.66}$	8	1+++ (9); 1? (28)
B10	10	$10^{-6.00}$	9	1+++ (17)
B20	10	$10^{-5.50}$	9	1+ (19)
Total	80		70	10

+ = 1 limb paralyzed; ++ = 2 limbs paralyzed; +++ = 3 or 4 limbs paralyzed; ++++ = prostrate.

*Days after inoculation.

to $10^{-6.00}$. All inoculated monkeys were kept under observation for at least 60 days. Summarizing, it is seen that, of a total of 80 rhesus monkeys inoculated intracerebrally, 70 remained normal, whereas 10 showed varying degrees of paralysis. The monkey marked questionable on the 28th day that received vaccine A40 showed transitory weakness lasting for only a day or so, but did not become paralyzed. It should be noted, however, that none of the paralyzed monkeys died.

It is of interest to compare these results with chick-embryo-propagated yellow fever vaccines, strain 17D. In a series of 200 rhesus monkeys inoculated intracerebrally with a number of yellow fever vaccine preparations, in which the vaccines showed titers ranging from 20,000 to 2 million LD_{50} units for mice, 6% of the monkeys died from yellow fever encephalitis, and an additional 15% developed mild weakness or paralysis.[92] Thus, it would appear that the results obtained by intracerebral inoculation of rhesus monkeys with these early poliomyelitis vaccines are quite comparable to those obtained under similar conditions with yellow fever vaccines.

Table II shows the results obtained when chimpanzees were inoculated either orally with 5 ml each or intramuscularly with 2 ml each of chick embryo vaccine A17. None of the chimpanzees showed any ill effects following vaccination. Blood samples were collected and tested for neutralizing antibodies on the days indicated. Chick embryo vaccine A17 showed a mouse titer of $10^{-5.5}$ LD_{50}. Neutralization tests were carried out with homologous MEF1 virus of the 131st hamster passage. The dosages used for vaccination were considered to be practical quantities for human inoculation. On the 127th day postvaccination, all four chimpan-

TABLE II. Neutralization Indices of Chimpanzees Receiving Chick Embryo MEF1–17 A Virus (Mouse Titer $10^{-5.5}$); Virus Used: MEF1 Hamster-Adapted 131 A)

Chimpanzee	Route of Inoculation	Interval in Days				
		14	42	113	127*	141
Carl	5 cc oral	12,590	2,140	31,620	11,480	12,590
Lucy	5 cc oral	3,160	1,075	1,905	8,510	5,130
Banjo	2 cc IM	4,365	680	33,880	1,820	70,790
Homer	2 cc IM	14,790	2,140	17,380	210	1,780
Virus titer of test		$10^{-8.5}$	$10^{-7.33}$	$10^{-7.0}$	$10^{-6.76}$	$10^{-6.85}$

*Second 5-cc administration by oral route.

zees were revaccinated orally with 5 ml each of the same vaccine. It is seen that the two chimpanzees initially inoculated orally gave as good neutralization titers, if not better, than those initially injected intramuscularly. The two chimpanzees initially injected intramuscularly showed a good rise in antibody titer following their oral reinoculation on the 127th day.

As stated previously, the data presented are by necessity preliminary in nature, but they are indicative of the general trend of our studies.

In summary, I wish to emphasize that no one, at the present time, can predict when a practical and satisfactory vaccine will become available for use. To attempt to make such a prediction at this time would be folly because a tremendous amount of work yet remains to be done. However, the tools for accurate, quantitative work are now available to accomplish the task. My colleagues and I are more confident than ever that eventually all three major types of poliomyelitis virus will be grown in the developing chick embryo. We believe that a living, attenuated virus vaccine, comprised of all three major types of poliomyelitis virus propagated in chick embryos and administered by the oral route, offers the most hopeful, practical, and safe procedure to follow for the immunization of children.

REFERENCES

1. Cox HR. Viral vaccines and human welfare. *Lancet*. 1953;265:1–5.
2. Morgan IM. Immunization of monkeys with formalin-inactivated poliomyelitis viruses. *Am J Hyg*. 1948;48:394–406.
3. Howe HA. Antibody response of chimpanzees and human beings to formalin-inactivated trivalent poliomyelitis vaccine. *Am J Hyg*. 1952;56:265–286.
4. Koprowski H, Cox HR. Studies on chick embryo adapted rabies virus; culture characteristics and pathogenicity. *J Immunol*. 1948;60:533–554.
5. Koprowski H. Experimental studies on rabies virus. *Can J Public Health*. 1949;40:60–67.
6. Cox HR. Review of chick-embryo adapted living rabies virus vaccines, with primary emphasis on use in dogs. *Proc US Live Stock Sanit Assoc*. 1949;53:264–272.

7. Koprowski H, Black J. Studies on chick-embryo adapted rabies virus; pathogenicity for dogs and use of egg-adapted strains for vaccination purposes. *J Immunol.* 1950;64: 185–196.
8. Koprowski H. Immunization with modified living vvirus with particular reference to rabies and hog cholera. *Vet Med.* 1952;47:144–150, 154.
9. Koprowski H, Black J. Studies on chick embryo adapted rabies virus; duration of immunity in vaccinated dogs. *Proc Soc Exp Biol Med.* 1952;80:410–415.
10. Schroeder CR, Black J, Burkhart RL, Koprowski H. Rabies in cattle; prevention of vampire bat paralytic rabies, derriengue, by vaccination with chick-embryo-adapted rabies virus. *Vet Med.* 1952;47:502–506.
11. Enders JF, Weller TH, Robbins FC. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science.* 1949;109:85–87.
12. Weller TH, Robbins FC, Enders JF. Cultivation of poliomyelitis virus in cultures of human foreskin and embryonic tissues. *Proc Soc Exp Biol Med.* 1949;72:153–155.
13. Robbins FC, Enders JF, Weller TH. The effect of poliomyelitis virus upon cells in tissue cultures [abstract]. *J Clin Invest.* 1950;29:841.
14. Robbins FC, Enders JF, Weller TH, Florentino GL. Studies on the cultivation of poliomyelitis viruses in tissue culture; the direct isolation and serologic identification of virus strains in tissue culture from patients with nonparalytic and paralytic poliomyelitis. *Am J Hyg.* 1951;54:286–293.
15. Enders JF. General preface to studies on the cultivation of poliomyelitis viruses in tissue cultures. *J Immunol.* 1952;69:639–643.
16. Weller TH, Enders JF, Robbins FC, Stoddard MB. Studies on the cultivation of poliomyelitis viruses in tissue culture; the propagation of poliomyelitis viruses in suspended cell cultures of various human tissues. *J Immunol.* 1952;69:645–671.
17. Robbins FC, Weller TH, Enders JF. Studies on the cultivation of poliomyelitis viruses in tissue culture; the propagation of the poliomyelitis viruses in roller-tube cultures of various human tissues. *J Immunol.* 1952;69:673–694.
18. Svedmyr A, Enders JF, Holloway A. Complement fixation with Brunhilde and Lansing poliomyelitis viruses propagated in tissue culture. *Proc Soc Exp Biol Med.* 1952;79: 296–300.
19. Svedmyr A, Enders JF, Holloway A. Complement fixation with the three types of poliomyelitis viruses propagated in tissue culture. *Am J Hyg.* 1953;57:60–70.
20. Youngner JS, Ward EN, Salk JE. Studies on poliomyelitis viruses in cultures of monkey testicular tissues; propagation of virus in roller tubes. *Am J Hyg.* 1952;55:291–300.
21. Youngner JS, Ward EN, Salk JE. Studies on poliomyelitis viruses in cultures of monkey testicular tissue; differences among strains in tissue culture infectivity with preliminary data on the quantitative estimation of virus and antibody. *Am J Hyg.* 1952;55:301–322.
22. Youngner JS, Lewis LJ, Ward EN, Salk JE. Studies on poliomyelitis viruses in cultures of monkey testicular tissue; isolation and immunologic identification of poliomyelitis viruses from fecal specimens by means of roller tube cultures. *Am J Hyg.* 1952;55: 347–356.
23. Ledinko N, Riordan JT, Melnick JL. Differences in cellular pathogenicity of two immunologically related poliomyelitis viruses as revealed in tissue culture. *Proc Soc Exp Biol Med.* 1951;78:83–88.
24. Ledinko N, Melnick JL. Poliomyelitis viruses in tissue culture; susceptibility of testicular tissue from immune monkeys. *Proc Soc Exp Biol Med.* 1952;81:144–148.
25. Melnick JL, Riordan JT. Poliomyelitis viruses in tissue culture; protein-free nutrient media in stationary and roller tube cultures. *Proc Soc Exp Biol Med.* 1952;81:208–213.
26. Ledinko N, Riordan JT, Melnick JL. Multiplication of poliomyelitis viruses in tissue cultures of monkey testes; growth curves of Type 1 (Brunhilde) and Type 2 (Lansing) strains and description of a quantitative neutralization test. *Am J Hyg.* 1952;55:323–338.
27. Riordan JT, Ledinko N, Melnick JL. Multiplication of poliomyelitis viruses in tissue cultures of monkey testes; direct isolation and typing of strains from human stools and spinal cords in roller tubes. *Am J Hyg.* 1952;55:339–346.

28. Syverton JT, Scherer WF, Butorac G. Propagation of poliomyelitis virus in cultures of monkey and human testicular tissues. *Proc Soc Exp Biol Med.* 1951;77:23–28.
29. Syverton JT, Scherer WF. Studies on the propagation *in vitro* of poliomyelitis viruses; viral multiplication in tissue cultures employing monkey and human testicular cells. *J Exp Med.* 1952;96:355–367.
30. Scherer WF, Syverton JT. Studies on the propagation *in vitro* of poliomyelitis viruses; a description of the growth curve and its relation to the cytopathogenicity of poliomyelitis virus. *J Exp Med.* 1952;96:369–388.
31. Scherer WF, Syverton JT. Studies on the propagation *in vitro* of poliomyelitis viruses; the propagation of poliomyelitis viruses in tissue cultures devoid of nerve cells. *J Exp Med.* 1952;96:389–400.
32. Scherer WF, Syverton JT, Gey GO. Studies on the propagation *in vitro* of poliomyelitis viruses; viral multiplication in a stable strain of human malignant epithelial cells (strain Héla) derived from an epidermoid carcinoma of the cervix. *J Exp Med.* 1953;97:695–710.
33. Franklin AE, Duncan D, Wood W, Rhodes AJ. Cultivation of Lansing poliomyelitis virus in tissue culture; utilization of glucose in synthetic medium. *Proc Soc Exp Biol Med.* 1952;79:715–718.
34. Thicke JC, Duncan D, Wood W, Franklin AE, Rhodes AJ. Cultivation of poliomyelitis virus in tissue culture; growth of the Lansing strain in human embryonic tissues. *Can J Med Sci.* 1952;30:231–245.
35. Wood W, Franklin AE, Clark EM, Duncan D, Rhodes AJ. Cultivation of poliomyelitis virus in tissue culture; synthetic medium in roller tube cultures. *Proc Soc Exp Biol Med.* 1952;81:434–438.
36. Smith WM, Chambers VC, Evans CA. Growth of neurotropic viruses in extraneural tissues; preliminary report on the propagation of poliomyelitis virus (Lansing and Hof. strains) in cultures of human testicular tissue. *Northwest Med.* 1950;49:368–373.
37. Smith WM, Chambers VC, Evans CA. Growth of neurotropic viruses in extraneural tissues; poliomyelitis virus in human testicular tissue *in vitro*. *Proc Soc Exp Biol Med.* 1951;76:696–700.
38. Salk JE. Studies in human subjects on active immunization against poliomyelitis; a preliminary report on experiments in progress. *JAMA.* 1953;151:1081–1098.
39. Salk JE. Use of adjuvants in studies on influenza immunization; degree of persistence of antibody in human subjects two years after vaccination. *JAMA.* 1953;151:1169–1175.
40. Eckardt RE, Director, Medical Research Division, Esso Laboratories, Linden, NJ. Personal communication; 1953.
41. Twort CC, Twort JM. The carcinogenic potency of mineral oils. *J Ind Hyg.* 1931;13: 204–226.
42. Page RC, de Beer EJ. Production of cysts following intramuscular injection of vegetable oils. *Am J Med Sci.* 1943;205:812–814.
43. Ehrich WE, Halbert SP, Mertens E, Mudd S. Mechanism of the augmenting action of mineral oil on antibody production; tissue reactions and antibody response to dysentery vaccine in saline and in saline-lanolin-mineral-oil emulsion. *J Exp Med.* 1945;82:343–360.
44. Hueper WC, Chief, Environmental Cancer Section, National Cancer Institute, Bethesda, Md. Personal communication; 1953.
45. Pait CF, Pearson HE. Rabies vaccine encephalomyelitis in relation to the incidence of animal rabies in Los Angeles. *Am J Public Health.* 1949;39:875–877.
46. Remlinger P, Bailly J. Paralysies au cours du traitement antirabique chez le chien; application possible à Pétude des accidents paralytiques de l'homme. *C R Soc Biol.* 1927;96:772–773.
47. Gey GO, Coffman WD, Kubicek MT. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium [abstract]. *Cancer Res.* 1952;12:264–265.
48. Li CP, Schaeffer M. Adaptation of Type 1 poliomyelitis virus to mice. *Proc Soc Exp Biol Med.* 1953;82:477–481.
49. Theiler M, Smith HH. The use of yellow fever virus modified by *in vitro* cultivation for human immunization. *J Exp Med.* 1937;65:787–800.

50. Theiler M, Smith HH. The effect of prolonged cultivation *in vitro* upon the pathogenicity of yellow fever virus. *J Exp Med.* 1937;65:767–786.
51. Theiler M. *The Development of Vaccines Against Yellow Fever.* (Les Prix Nobel en 1951). Stockholm: Kungl. Boktryckeriet P.A. Norstedt and Söner; 1952.
52. Cox HR. Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and typhus groups. *Public Health Rep.* 1938;53:2241–2247.
53. Cox HR. A filter-passing infectious agent isolated from ticks; description of organism and cultivation experiments. *Public Health Rep.* 1938;53:2270–2276.
54. Cox HR, Bell EJ. The cultivation of Rickettsia diaporica in tissue culture and in the tissues of developing chick embryos. *Public Health Rep.* 1939;54:2171–2178.
55. Cox HR. Rocky Mountain spotted fever; protective value for guinea pigs of vaccine prepared from rickettsiae cultivated in embryonic chick tissues. *Public Health Rep.* 1939; 54:1070–1077.
56. Cox HR. Rickettsia diaporica and American Q fever. *Am J Trop Med.* 1940;20:463–469.
57. Cox HR. Cultivation of rickettsiae of the Rocky Mountain spotted fever, typhus and Q fever groups in the embryonic tissues of developing chicks. *Science.* 1941;94:399–403.
58. Cox HR. Some recent advances and current problems in the field of rickettsial diseases. *Arch Ges Virusforsch.* 1951;4:518–533.
59. Penna HA, Moussatché H. Modificação do virus de febre amarella por passagens em serie no embryão de gallinha em desenvolvimento. *Brasilmed.* 1939;53:903–904.
60. Cox HR. Growth of viruses and rickettsiae in the developing chick embryo. *Ann NY Acad Sci.* 1952;55:236–247.
61. Cox HR. Tissue cultures as a more sensitive method than animal inoculation for detecting equine encephalomyelitis virus. *Proc Soc Exp Biol Med.* 1936;33:607–609.
62. Frenkel HS, Frederiks HHJ. Cultivation of foot-and-mouth disease virus in explanted epithelial tissue of the bovine rumen. *Nature.* 1949;164:235–236.
63. Frenkel HS. Histologic changes in explanted bovine epithelial tongue tissues infected with the virus of foot-and-mouth disease. *Am J Vet Res.* 1949;10:142–145.
64. Frenkel HS, Dunne HW, Osteen OL. Foot-and-mouth disease virus propagation. *J Am Vet Med Assoc.* 1949;115:178.
65. Frenkel HS. Research on foot-and-mouth disease; the cultivation of the virus on a practical scale in explantations of bovine tongue epithelium. *Am J Vet Res.* 1951;12: 187–190.
66. Frenkel HS, Dunne HW. Research on foot-and-mouth disease; cultivation of the foot-and-mouth disease virus in explanted epithelium of the bovine tongue; tissue and medium variations. *Am J Vet Res.* 1952;13:21–23.
67. Boynton WH. Preliminary report on the propagation of hog cholera virus *in vitro. Vet Med.* 1946;41:346–347.
68. Boynton WH, Takahashi WN, Woods GM, Walker WW. Further studies on propagation of hog cholera virus *in vitro* including electron micrographs. *Vet Med.* 1948;43:403–406.
69. Clancy CF, Cox HR, Bottorff CA. Laboratory experiments with living Newcastle disease vaccine. *Poult Sci.* 1949;28:58–62.
70. Markham FS, Cox HR, Bottorff CA. Field trials with living virus vaccine for Newcastle disease. *Poult Sci.* 1949;28:52–57.
71. Markham FS, Bottorff CA, Tennison LB, Jr. Observations on the relationship of passive immunity to response following intranasal vaccination against Newcastle disease. *Proc US Live Stock Sanit Assoc.* 1950;54:161–165.
72. Markham FS, Bottorff CA, Cox HR. The conjunctival application of Newcastle disease vaccine (intranasal type) in parentally immune and susceptible chicks. *Cornell Vet.* 1951;41:267–282.
73. Cabasso V, Cox HR. Propagation of canine distemper virus on the chorio-allantoic membrane of embryonated hen eggs. *Proc Soc Exp Biol Med.* 1949;71:246–250.
74. Cabasso VJ, Burkhart RL, Leaming JD. The use of an egg-adapted modified canine

distemper virus vaccine under experimental conditions and in the field. *Vet Med.* 1951; 46:167–175, 179.

75. Cabasso VJ, Cox HR. A distemperlike strain of virus derived from a case of canine encephalitis; its adaptation to the chick embryo and subsequent modification. *Cornell Vet.* 1952;42:96–107.
76. Cabasso VJ. Canine distemper and "hard pad" disease. *Vet Med.* 1952;47:417–423.
77. Cabasso VJ. Canine viruses; distemper and "hard pad" disease, infectious canine hepatitis and rabies control. *Southwest Vet.* 1952–1953;6:19–24, 137–141.
78. Cabasso VJ, Stebbins MR, Cox HR. Onset of resistance and duration of immunity to distemper in ferrets following a single injection of avianized distemper vaccine. *Vet Med.* 1953;48:147–150.
79. Koprowski H, James TR, Cox HR. Propagation of hog cholera virus in rabbits. *Proc Soc Exp Biol Med.* 1946;63:178–183.
80. Koprowski H, James TR, Cox HR. Laboratory studies and data on modified live hog cholera vaccine. *Proc US Live Stock Sanit Assoc.* 1951;55:214–223.
81. Schroeder CR, Harvey MJ, Koprowski H, James TR, Cox HR. Trends in hog cholera immunization. *Southwest Vet.* 1952;5:217–223.
82. Harvey MJ, Burkhart RL, Leaming JD, Percival RC, Schroeder CR, Welsh M. Field trial studies with Rovac. *Proc US Live Stock Sanit Assoc.* 1951;55:224–279.
83. Gear J. Immunity to poliomyelitis. *Ann Intern Med.* 1952;37:1–22.
84. Kolmer JS, Rule AM. Concerning vaccination of monkeys against acute anterior poliomyelitis with special reference to oral immunization. *J Immunol.* 1934;26:505–515.
85. Koprowski H, Jervis GA, Norton TW. Immune responses in human volunteers upon oral administration of a rodent-adapted strain of poliomyelitis virus. *Am J Hyg.* 1952; 55:108–126.
86. Koprowski H, Jervis GA, Norton TW, Nelsen DJ. Further studies on oral administration of living poliomyelitis virus to human subjects. *Proc Soc Exp Biol Med.* 1953;82:277–280.
87. Roca-Garcia M, Moyer AW, Cox HR. Poliomyelitis; propagation of MEF1 strain of poliomyelitis virus in developing chick embryo by yolk sac inoculation. *Proc Soc Exp Biol Med.* 1952;81:519–525.
88. Cabasso VJ, Stebbins MR, Dutcher RM, Moyer AW, Cox HR. Poliomyelitis; propagation of MEF1 strain of poliomyelitis virus in developing chick embryo by allantoic cavity inoculation. *Proc Soc Exp Biol Med.* 1952;81:525–529.
89. Moyer AW, Accorti C, Cox HR. Poliomyelitis; propagation of the MEF1 strain of poliomyelitis virus in the suckling hamster. *Proc Soc Exp Biol Med.* 1952;81:513–518.
90. Roca-Garcia M. et al. Unpublished data.
91. Cabasso VJ. et al. Unpublished data.
92. Burruss H. US government technical consultant on yellow fever vaccine, National Drug Co., Swiftwater, Pa. Personal communication; 1953.